Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Emergence of Q493R mutation in SARS-CoV-2 spike protein during bamlanivimab/etesevimab treatment and resistance to viral clearance

Dear Editor,

Data from clinical trials suggest that monoclonal antibody (mAb) treatments can prevent deaths and severe disease among people with mild to moderate COVID-19. Recently, a cocktail of two mAbs- bamlanivimab and etesevimab- was reported to cut the risk of hospitalization and death by 87%.[1] The European Medicine Agency (EMA) recommends the use of this combination in patients who do not require supplemental oxygen, and who are at high risk of progression to severe disease.[2] Since SARS-CoV-2 Spike protein is the target of antibody-based therapeutics, they all suffer from one major risk: mutational escape of the Spike protein.[3]

We here report a well-characterized case of the emergence of SARS-CoV-2 Spike escape.

Q493R mutation during bamlanivimab/etesevimab treatment.

A 63-year-old patient was diagnosed in July 2017 with cutaneous T-cell lymphoma (mycosis fungoides). The patient underwent several treatments including carmustine, methotrexate, bexarotene, and extracorporeal photopheresis. Tumoral extension to lymph nodes (stage III) led to a CHOEP chemotherapy regimen. An allogeneic hematopoietic stem cell transplantation was then performed in January 2021, complicated by the occurrence of a graft-versus-host disease.

A COVID-19 screening was performed in April 2021, due to an intrafamilial exposure, and a positive RT-PCR result was found on a nasopharyngeal (NP) specimen. The patient developed mild symptoms such as runny nose and dry cough. A treatment with bamlanivimab (700 mg) /etesevimab (1400 mg) was initiated. Sequential NP samples were collected for treatment follow-up.

SARS-CoV-2 RNA detection and E484K mutation screening were performed with the AllplexTM SARS-CoV-2 Variants I Assay (Seegene, Eurobio®). For the viral whole genome sequencing, total RNA extraction was performed using the MGIEasy Nucleic Acid Extraction Kit on the MGISP-960 instrument (BGI®). The libraries were prepared using Illumina® COVIDSeq protocol, and paired-end sequencing with 150 bp read length was carried out on NextSeq 550 platform. Data were processed using DRAGEN COVIDSeq Test Pipeline 1.0.0 (Illumina®). Clades and lineages were assigned to the genomes according to the Nextstrain nomenclature (version 1.0.0) and the PANGOLIN package (version 2021.06.15), respectively. Mutation analysis of the Spike region was provided by Nextstrain and GISAID CoVsuver tool.

Serum samples were also collected for antibody testing. Qualitative detection of anti-S antibodies was done with the Wantai SARS-CoV-2 Ab ELISA kit (Eurobio®) and the Quantivac.

ELISA Anti SARS-CoV-2 (Euroimmun®) was used for antibody quantification.

Overall, the clinical evolution was good regarding COVID-19. The patient was discharged from hospital just after the initiation of bamlanivimab/etesevimab treatment which was well tolerated, and was followed as outpatient in the infectious disease department. She experienced prolonged respiratory symptoms with dyspnea and cough; however, no worsening of the status was observed and patient did not require oxygen therapy or hospitalization for COVID-19 symptoms. Furthermore, chest X-ray and biological inflammatory markers remained normal.

Virological data are summarized in Table 1, and show that the viral clearance was not efficient, with a viral load which remains high almost 40 days after the initiation of treatment. Viral whole genome sequencing identified the widely circulating B.1.1.7 lineage (20I/501Y.V1) SARS-CoV-2. The spike mutation analysis did not detect the E484K mutation during the follow-up; however, an A → G mutation was observed at nucleotide position 23,040 from day 15 post-treatment leading to Q493R amino-acid substitution in the spike protein. Good quality sequences were obtained in all tested samples, with a genome coverage between (99.99 and 100%), and a median number of reads per nucleotidic position between 174 and 3465. In addition, a focus on the specific position showed a very good coverage between 142X and 2516X (See Table 2).

Furthermore, antibody testing confirmed the presence of high levels of anti-Spike IgG antibodies.

This observation is clearly compatible with a reduced efficacy or a lack of efficacy of the bamlanivimab/etesevimab treatment. In the BLAZE-1 trial (phase 2), a combination treatment with 2800 mg of bamlanivimab and 2800 mg of etesevimab led to viral load change of −4.37 and −17.91 from baseline to day 11 and day 29 post-treatment, respectively. A Ct value lower than 27.5 on day 7 post-treatment was considered as persistently high viral load.[1] The resistance to treatment observed is this case is probably associated with the Q439R substitution that emerged in the Spike protein. The high initial viral load and the viral on-treatment replication could have contributed to the occurrence of this mutation.

Changes in the spike protein can significantly alter the efficacy of mAbs, and the most dangerous for immune escape are the ones occurring within the receptor binding domain (RBD).[3]

In-vitro studies have identified mainly six amino-acid substitutions corresponding to four positions (E484D/K/Q, F490S, Q493R, and S494P) that led to a reduced susceptibility to bamlanivimab, while six amino-acid substitutions at three positions (K417N, D420N, and N460K/S/T/Y) were shown to be critical for etesevimab. More interestingly, a retention of susceptibility to the other antibody alone was observed, with the exception of the Q493R substitution. A pseudotyped virus-like particle assay showed for variants harboring E484K, E484Q, and Q493R substitutions, a re-

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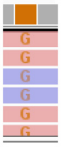
Table 1
Virological data during treatment follow-up.

Virological data	Bamlanivimab/Etesevimab treatment						
Day post treatment	−2 (diagnosis)	0	7	15	21	27	39
CT value (RDRP gene, Allplex assay)	18.6	9.9	20.6	15.5	15.1	14.3	24.8
SARS-CoV-2 lineage	NA	B.1.1.7	B.1.1.7	B.1.1.7	B.1.1.7	NA	B.1.1.7
Presence of E484K/Q mutation (Yes/No)	No	No	No	No	No	No	No
Amino-acid at Spike position 493	NA	Q	Q	R	R	NA	R
Number of reads at Spike nucleotide position 23,040 (total number of reads)	NA	177A (177)	142A (142)	169 G (169)	2506 G (2516)	NA	935 G (936)
GISAID reference for viral genome sequence	NA	EPI_ISL_ 2,143,435	EPI_ISL_ 2,227,230	EPI_ISL_ 2,361,537	EPI_ISL_ 2,444,359	NA	EPI_ISL_ 2,646,207
Total antibody testing (qualitative, Wantai) (Index)	NA	NA	11.6	13	NA	10.3	12.5
IgG quantification (Euroimmun) (binding activity units/mL)	NA	NA	>1920	>1920	NA	>1920	>1920

Table 2
A→G mutation at nucleotidic position 23,040.

Day post-treatment	GISAID sequence reference	Nucleotide position 23040	Detailed number of reads
0	EPI_ISL_2143435		NC_045512.2:23 040 Total count: 177 A : 177 (100%, 85+, 92-) C : 0 G : 0 T : 0 N : 0
7	EPI_ISL_2227230		NC_045512.2:23 040 Total count: 142 A : 142 (100%, 62+, 80-) C : 0 G : 0 T : 0 N : 0
15	EPI_ISL_2361537		NC_045512.2:23 040 Total count: 169 A : 0 C : 0 G : 169 (100%, 78+, 91-) T : 0 N : 0
21	EPI_ISL_2444359		NC_045512.2:23 040 Total count: 2516 A : 8 (0%, 5+, 3-) C : 1 (0%, 0+, 1-) G : 2506 (100%, 1042+, 1464-) T : 1 (0%, 0+, 1-)
39	EPI_ISL_2646207		NC_045512.2:23 040 Total count: 936 A : 0 C : 0 G : 935 (100%, 353+, 582-) T : 1 (0%, 0+, 1-) N : 0

duced susceptibility to bamlanivimab and etesevimab combination of 17-fold, 22-fold, and > 100-fold, respectively.[2,4]

The escape site Q493 seems thus to be the most critical for the bamlanivimab and etesevimab cocktail. It was recently reported that this site is not in the receptor-binding ridge, but in a region of joint structural overlap by both antibodies, such that a substitution leading to a positively charged residue (R, K) may directly affect binding by each antibody.[5]

As we prepare this manuscript, another team also described a similar case using Sanger sequencing of the viral spike region, with a follow-up period of 2 weeks.[6]

In conclusion, the emergence of the spike protein Q493R mutation can lead to virological failure during bamlanivimab/etesevimab treatment, and should be rapidly screened in nonresponders, especially when immunocompromized.

Ethical statement

This report was approved by the Institutional data protection authority of CHU Lille. Informed consent was obtained from the patient and data have been anonymized as much as possible.

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